FEBS Letters 585 (2011) 3898–3902

FEBS Letters

journal homepage: www.FEBSLetters.org

Streptomyces erythraeus trypsin inactivates α_1-antitrypsin

Krishna M. Vukoti [a], Chandra Sekhar Rao Kadiyala [a,1], Masaru Miyagi [a,b,c,*]

[a] Case Center for Proteomics and Bioinformatics, Case Western Reserve University, 10900 Euclid Ave., Cleveland, OH 44106, USA
[b] Department of Pharmacology, Case Western Reserve University, 10900 Euclid Ave., Cleveland, OH 44106, USA
[c] Department of Ophthalmology and Visual Sciences, Case Western Reserve University, 10900 Euclid Ave., Cleveland, OH 44106, USA

ARTICLE INFO

Article history:
Received 5 September 2011
Revised 6 November 2011
Accepted 8 November 2011
Available online 21 November 2011

Edited by Judit Ovádi

Keywords:
Streptomyces erythraeus trypsin (SET)
Trypsin
α_1-Antitrypsin
Serpin

ABSTRACT

***Streptomyces erythraeus* trypsin (SET) is a serine protease that is secreted extracellularly by *S. erythraeus*. We investigated the inhibitory effect of α_1-antitrypsin on the catalytic activity of SET. Intriguingly, we found that SET is not inhibited by α_1-antitrypsin. Our investigations into the molecular mechanism underlying this observation revealed that SET hydrolyzes the Met–Ser bond in the reaction center loop of α_1-antitrypsin. However, SET somehow avoids entrapment by α_1-antitrypsin. We also confirmed that α_1-antitrypsin loses its inhibitory activity after incubation with SET. Thus, our study demonstrates that SET is not only resistant to α_1-antitrypsin but also inactivates α_1-antitrypsin.**

Structured summary of protein interactions:
BT cleaves **alpha1 antitrypsin** by protease assay (View interaction)
alpha1 antitrypsin and **BT** bind by comigration in non denaturing gel electrophoresis (View interaction)
SET cleaves **alpha1 antitrypsin** by protease assay (View interaction)

1. Introduction

A serine protease, *Streptomyces erythraeus* trypsin (SET), was first isolated by Yoshida and coworkers four decades ago from the culture broth of *S. erythraeus* [1]. SET specifically hydrolyzes both lysyl and arginyl peptide bonds using a catalytic mechanism that is essentially the same as that of trypsins from mammalian sources [2,3]. As expected, SET is inhibited by substrate-analogue inhibitors, tosyl-Lys-CH_2Cl and tosyl-Arg-CH_2Cl [4], and by a Kazal-type trypsin inhibitor, ovomucoid [5,6]. The mature SET consists of 227 amino acid residues, with molecular weight of 22.3 kDa. SET shares 33% amino acid sequence identity with bovine trypsin (BT) [2] and folds to essentially the same tertiary structure as the mammalian enzyme [7]. However, there are two notable differences between SET and the mammalian trypsins. Unlike the mammalian protein, SET is resistant to autolysis due to the absence of an arginine or lysine residue in the so-called "autolysis loop" [2,8,9]. Another unique feature of SET is that the catalytic activity level (K_m/k_{cat}) for N^{α}-benzoyl-L-arginine-*p*-nitroanilide is more than one order of magnitude higher than that of BT [1,9].

We suspected that the bacterial protease SET would not be inhibited by a serum serine protease inhibitor, such as α_1-antitrypsin, because SET is not of mammalian origin. Therefore, we tested whether α_1-antitrypsin inhibits SET. α_1-Antitrypsin is a 52-kDa secreted glycoprotein of the serpin (serine protease inhibitor) protein superfamily [10]. Its major function is the inhibition of neutrophil elastase [11], but it also inhibits other proteases, including trypsin. α_1-Antitrypsin has a reactive center loop that functions as 'bait' for a target protease. Following the formation of a Michaelis–Menten complex and the cleavage of the Met358–Ser359 bond in the reactive center loop, α_1-antitrypsin undergoes an extensive conformational rearrangement that traps the protease before completion of its catalytic cycle at the acylenzyme intermediate stage [12,13]. Because the acylenzyme intermediate is hydrolyzed extremely slowly [13], the inhibition of the trapped protease is virtually irreversible.

We studied the inhibitory effect of α_1-antitrypsin on the catalytic activity of SET and the interaction between SET and α_1-antitrypsin. We report here that SET does hydrolyze the Met–Ser

Abbreviations: Bz-Arg-*p*NA, N^{α}-benzoyl-L-arginine-*p*-nitroanilide; BT, bovine trypsin; IPTG, isopropyl β-D-thiogalactoside; MALDI-TOF MS, matrix assisted laser desorption ionization time of flight mass spectrometry; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethanesulfonylfluoride; SDS, sodium dodecyl sulfate; SET, *Streptomyces erythraeus* trypsin; TPCK, N^{α}-*p*-tosyl-L-phenylalanine chloromethyl ketone

* Corresponding author at: Case Center for Proteomics and Bioinformatics, Case Western Reserve University, 10900 Euclid Ave., BRB 928, Cleveland, OH 44106-4988, USA. Fax: +1 216 368 6846.

E-mail addresses: mxm356@case.edu, masaru.miyagi@case.edu (M. Miyagi).

[1] Current address: Department of Cell Biology, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44195, USA.

0014-5793/$36.00 © 2011 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2011.11.015

bond in the reactive center loop of α_1-antitrypsin but is not inhibited by α_1-antitrypsin. The possible molecular mechanisms underlying this phenomenon are discussed.

2. Materials and methods

2.1. Materials

N^{α}-benzoyl-L-arginine-*p*-nitroanilide (Bz-Arg-*p*NA) was purchased from Bachem Americas (Torrance, CA), and isopropyl β-D-thiogalactoside (IPTG) from Promega (Madison, WI). Bovine trypsin, soybean trypsin inhibitor, human α_1-antitrypsin, phenylmethanesulfonylfluoride (PMSF), N^{α}-*p*-tosyl-L-phenylalanine chloromethyl ketone (TPCK) and *p*-aminobenzamidine-agarose were purchased from Sigma–Aldrich (St. Louis, MO). Precast gels for native and SDS–PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) were purchased from Bio-Rad (Hercules, CA). All other chemicals and materials used were either reagent grade or of the highest quality commercially available.

2.2. Preparation of recombinant SET

Cloning, expression, and purification of SET were conducted as described previously [9]. Briefly, the pET19b-SET plasmid that encodes his-tagged recombinant SET protein was transformed into *Escherichia coli* BL21-AI (Invitrogen, Carlsbad, CA), and protein expression was induced by the addition of 0.2% (w/v) arabinose and 1 mM isopropyl β-D-thiogalactoside (IPTG). After 5 h of induction, the cells were harvested by centrifugation and the cell pellet stored at −80 °C until used for protein purification. The harvested cells were lysed, and then the recombinant SET protein was purified by sequential affinity chromatography using the PrepEase Histidine-tagged Protein Purification Maxi Kit-High Specificity (USB Corporation, Cleveland, OH) and soybean trypsin inhibitor agarose beads (Pierce, Rockford, IL).

2.3. Inhibition assay of SET

SET (0.2 μM) was pre-incubated with 5 μM of either α_1-antitrypsin or soybean trypsin inhibitor (5 μM) in 100 mM ammonium bicarbonate at 25 °C for 10 min. After the incubation, a substrate, Bz-Arg-*p*NA (1 mM), was added to the solution and proteolytic activity was measured by monitoring the changes in absorbance (405 nm) at 25 °C with an Agilent UV/VIS spectrophotometer (Santa Clara, CA) [9]. The effect of the inhibitors against the activity of BT was also measured. The concentration of SET was determined by amino acid analysis performed at the Protein Chemistry Laboratory at Texas A&M University (College Station, TX) [9]. The concentrations of α_1-antitrypsin and BT were determined from their molar absorption coefficient values at 280 nm [14].

2.4. Native and SDS–PAGE analysis

Native and SDS–PAGE were carried out using a 4–20% Mini-PROTEAN precast gel (Bio-Rad). The native-PAGE sample buffer contained 25 mM Tris–HCl (pH 6.8), 10% glycerol, and 0.002% bromophenol blue. Protein samples were directly subjected to the native-PAGE. The SDS–PAGE sample buffer contained 25 mM Tris–HCl (pH 6.8), 2% SDS, 2% 2-mercaptoethanol, 10% glycerol, and 0.002% bromophenol blue. Protein samples were heated at 98 °C for 10 min prior to being subjected to the SDS–PAGE. The running buffer used for native-PAGE was 20 mM Tris–glycine (pH 8.3), and the running buffer for SDS–PAGE was 20 mM Tris–glycine (pH 8.3) containing 0.1% SDS. Proteins were visualized by staining with colloidal Coomassie Brilliant Blue G-250 (Bio-Rad).

2.5. Mass spectrometry analysis

The reaction of α_1-antitrypsin (40 pmol) with SET or BT (40 pmol), in 200 μL of 100 mM ammonium bicarbonate incubated at 25 °C for 30 s, was analyzed by MALDI-TOF MS (matrix assisted laser desorption ionization time of flight mass spectrometry) with a Perkin Elmer ProTOF 2000 mass spectrometer (Norwalk, CT). The matrix used was 5% (w/v) α-cyano-4-hydroxycinnamic acid in 0.1% (w/v) trifluoroacetic acid and 60% (v/v) acetonitrile. The matrix was mixed with sample solution at a 4:1 ratio by volume.

2.6. Inactivation assay of α_1-antitrypsin

α_1-Antitrypsin (700 pmol) was incubated with 700 pmol of SET in 100 μL of 100 mM ammonium bicarbonate at 25 °C for 10 min. After incubation, *p*-aminobenzamidine-agarose [15] was added to the reaction mixture to absorb the SET protein. The mixture was then centrifuged, and the resulting SET-free supernatant was subjected to the inhibition assay with BT and SDS–PAGE analysis, as described above.

3. Results

3.1. SET is resistant to α_1-antitrypsin

The inhibitory effects of α_1-antitrypsin and soybean trypsin inhibitor on the catalytic activity of SET were investigated and compared with inhibitory effects on BT, which was used as a positive control (Fig. 1a). The most striking finding was that SET activity was unaffected by incubation with α_1-antitrypsin, whereas it was completely lost following incubation with soybean trypsin

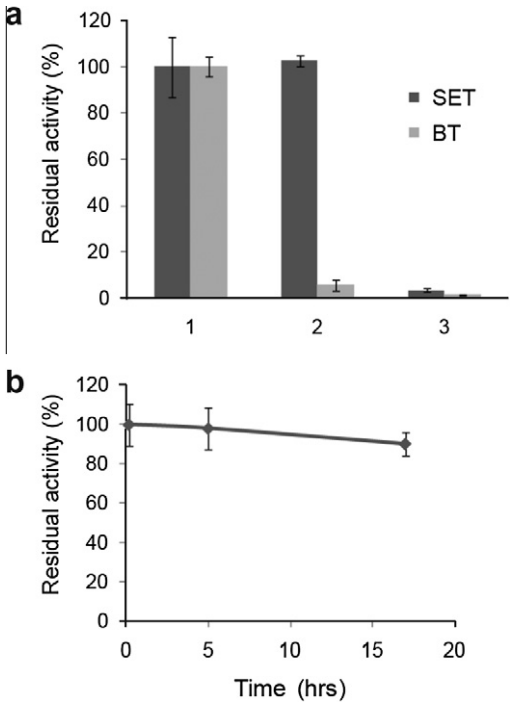

Fig. 1. Inhibition of SET by α_1-antitrypsin and soybean trypsin inhibitor. (a) SET (0.2 μM) or BT (0.2 μM) was incubated with α_1-antitrypsin (5 μM) or soybean trypsin inhibitor (5 μM) at 25 °C for 10 min, and residual catalytic activity was determined. Column 1: no inhibitor; column 2: α_1-antitrypsin; and column 3: soybean trypsin inhibitor. (b) SET (0.2 μM) was incubated with α_1-antitrypsin (5 μM) at 25 °C for 0, 5, and 17 h, and residual catalytic activity was determined. The values and error bars in the figure represent the means ± standard deviations from triplicate reactions.

inhibitor. These results indicate that SET is resistant to α_1-antitrypsin, but not to a Kunitz-type inhibitor like soybean trypsin inhibitor. As expected, BT was almost completely inhibited by either inhibitor. To eliminate the possibility of slow inhibition of SET by α_1-antitrypsin, we also incubated SET with α_1-antitrypsin for extended periods of time up to 17 h (Fig. 1b). SET retained greater than 90% of its activity after 17 h of incubation, indicating that SET is resistant to α_1-antitrypsin.

3.2. SET does not form a covalent complex with α_1-antitrypsin

It is well established that BT forms a covalent complex with α_1-antitrypsin and is thereby entrapped and inhibited [13]. Because SET is resistant to α_1-antitrypsin, it is unlikely that SET forms a covalent complex with α_1-antitrypsin. To experimentally verify this hypothesis, SET was incubated with an equimolar amount of α_1-antitrypsin for 1 min and then the mixture was analyzed by native-PAGE. As expected, no band corresponding to a SET–α_1-antitrypsin complex was observed (Fig. 2a, lane 3), whereas a band corresponding to a BT–α_1-antitrypsin complex was clearly visible, as indicated in lane 6 of Fig. 2a. This result shows that an equimolar amount of α_1-antitrypsin to SET does not yield appreciable amount of SET–α_1-antitrypsin complex. Note that the band that corresponds to BT was not observed in lane 5; this is because BT is a basic protein and therefore it did not migrate toward the anode under the pH condition used for the electrophoresis (pH 8.3).

To investigate whether SET–α_1-antitrypsin complex is formed when the molar ratio of α_1-antitrypsin to SET is increased, we incubated SET with varying amount of α_1-antitrypsin, and the reaction mixtures were subjected to native-PAGE and SET activity assay (Fig. 2b). A protein band that migrates slower than α_1-antitrypsin was not observed even at the α_1-antitrypsin/SET ratio of 100, indicating that SET does not form a covalent complex with α_1-antitrypsin. Consistent with the conclusion, essentially no reduction of SET band intensities and no loss of SET activities were observed up to the α_1-antitrypsin/SET ratio of 100 (Fig. 2b).

3.3. SET hydrolyzes the Met–Ser bond in the reactive center loop of α_1-antitrypsin

To explore the mechanism that would explain the α_1-antitrypsin resistance of SET, α_1-antitrypsin that had been incubated with SET was analyzed by SDS–PAGE to examine proteolysis of α_1-antitrypsin. Following incubation, two bands were visible other than the band of SET (Fig. 3a, lane 3). The upper faint band appeared to correspond to intact α_1-antitrypsin, and the lower intense band migrated as a protein of slightly lower molecular weight. Note that SET ran at an apparent molecular weight of 35 kDa, despite the fact that the actual molecular weight is 23.6 kDa. The reason for this is not clear; however this phenomenon has been reported [2,9]. Following the incubation of α_1-antitrypsin with BT, two bands were also observed (Fig. 3a, lane 6). The lower band appeared to have about the same molecular weight as the lower band in lane 3; suggesting that SET and BT hydrolyze the same site in α_1-antitrypsin, which is the Met358–Ser359 bond in the reactive center loop. The apparent molecular weight of upper band was slightly higher than that of α_1-antitrypsin, thus presumed to be the BT–α_1-antitrypsin complex.

To confirm the cleavage site on α_1-antitrypsin, the reaction solution of α_1-antitrypsin incubated with SET or BT was subjected to MALDI mass spectrometry. In both the reactions, a prominent peak corresponding to a peptide of molecular weight 4132.3 Da was observed (Fig. 3b), and this corresponds well to the molecular weight of the peptide (Ser359-Lys394, theoretical mass: 4132.2) produced by cleavage of the Met–Ser bond in the reactive center loop of α_1-antitrypsin. Thus, this result demonstrates that SET, like BT, hydrolyzes the Met358–Ser359 bond in the reactive center loop. The signal of truncated α_1-antitrypsin lacking Ser359-Lys394 was not observed, and this was assumed to be due to the heterogeneous nature of the glycosylations at Asn46, 83, and 247 [16].

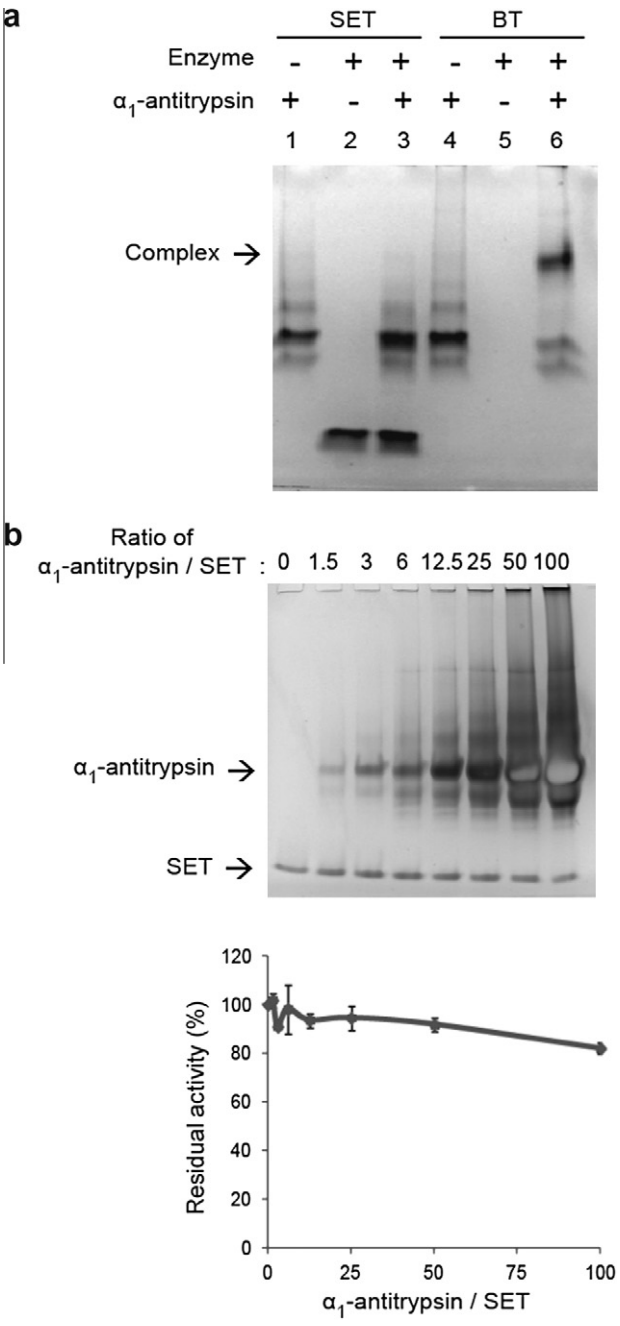

Fig. 2. Physical interaction of SET with α_1-antitrypsin. (a) SET or BT (60 pmol) was incubated with or without α_1-antitrypsin (60 pmol) in 100 μL of 100 mM ammonium bicarbonate at 25 °C for 10 min. The reaction was stopped by adding 100 μmol of PMSF and then subjected to native-PAGE. Lane 1: α_1-antitrypsin alone; lane 2: SET alone; lane 3: α_1-antitrypsin incubated with SET; lane 4: α_1-antitrypsin alone; lane 5: BT alone; lane 6: α_1-antitrypsin incubated with BT. (b) SET (20 pmol) was incubated with varying amount of α_1-antitrypsin (α_1-antitrypsin/SET ratios: 0–100) in 100 μL of 100 mM ammonium bicarbonate at 25 °C for 10 min. The reaction mixtures were then subjected to native-PAGE and SET activity assay. The results of activity assays are expressed as means ± standard deviations from triplicate reactions.

3.4. SET inactivates α_1-antitrypsin

It is known that cleavage within the reactive center loop sequence of α_1-antitrypsin by metalloproteinases inactivates the

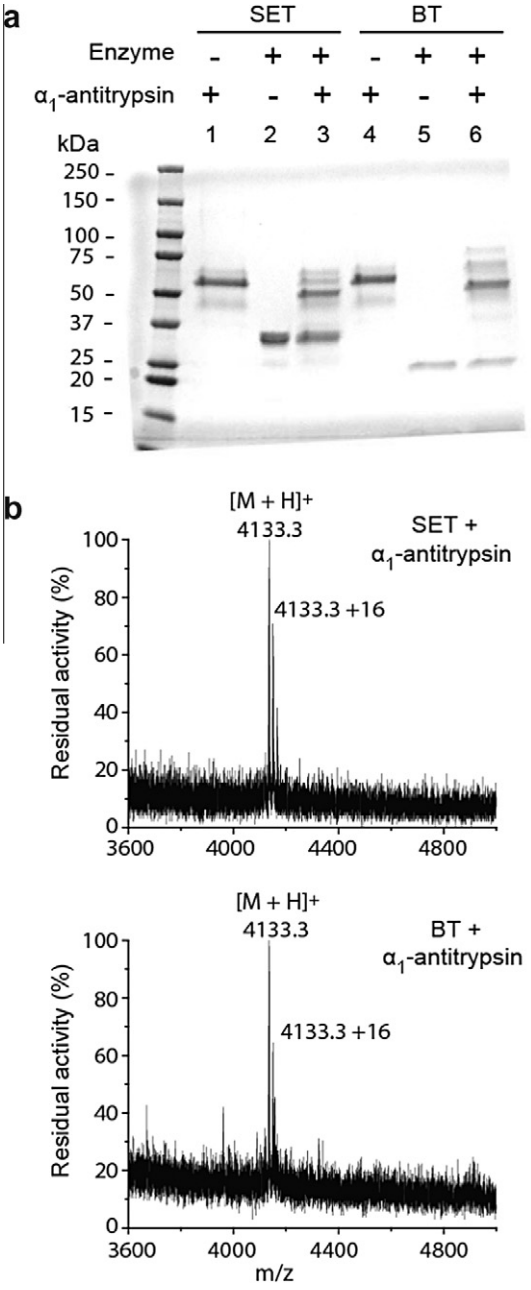

Fig. 3. Cleavage of α_1-antitrypsin by SET. (a) SET or BT (60 pmol) was incubated with or without human α_1-antitrypsin (60 pmol) in 100 μL of 100 mM ammonium bicarbonate at 25 °C for 1 min. The reaction was stopped by adding 100 μmol of PMSF and then subjected to SDS–PAGE. Lane 1: α_1-antitrypsin alone; lane 2: SET alone; lane 3: α_1-antitrypsin incubated with SET; lane 4: α_1-antitrypsin alone; lane 5: BT alone; lane 6: α_1-antitrypsin incubated with BT. (b) MALDI mass spectra of α_1-antitrypsin incubated with SET (upper panel) or BT (lower panel).

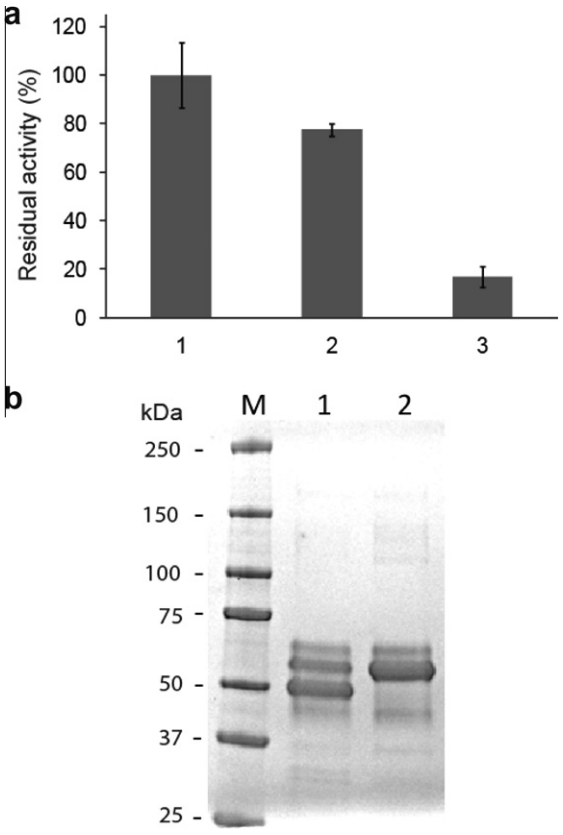

Fig. 4. Inactivation of α_1-antitrypsin by SET. α_1-Antitrypsin (700 pmol) was incubated with or without SET (700 pmol) in 100 μL at 25 °C for 10 min. After incubation, *p*-aminobenzamidine-agarose was added to the reaction mixture to remove the SET protein. The resulting SET-treated and -untreated α_1-antitrypsin was subjected to the inhibitory assay on the catalytic activity of BT and the SDS–PAGE analysis. (a) Inhibitory assay. Column 1: absence of α_1-antitrypsin control; column 2: SET-treated α_1-antitrypsin; column 3: SET-untreated α_1-antitrypsin. The results of activity assays are expressed as means ± standard deviations from triplicate reactions. (b) SDS–PAGE analysis. Lane 1: SET-treated α_1-antitrypsin; Lane 2: SET-untreated α_1-antitrypsin.

inhibitor [17–19]. To confirm that the cleavage at Met358–Ser359 in the reactive center loop of α_1-antitrypsin by SET causes loss of inhibition, SET-treated and -untreated α_1-antitrypsin were pre-incubated with BT and the residual tryptic activity was determined. As expected, BT activity was unaffected by SET-treated α_1-antitrypsin (Fig. 4a, column 2), but it was almost completely inhibited by a positive control untreated α_1-antitrypsin (Fig. 4a, column 3). SDS–PAGE analysis of the SET-treated and -untreated α_1-antitrypsin confirmed that the SET-treated α_1-antitrypsin was mostly cleaved form, and equal amount of SET-treated and -untreated α_1-antitrypsin were subjected to the inhibitory assay (Fig. 4a). Thus, these results are consistent with the inactivation of α_1-antitrypsin by hydrolysis of the Met358–Ser359 bond in the reactive center loop by SET.

4. Discussion

In this study, we have shown that SET is resistant to enzymatic inhibition by α_1-antitrypsin. To our knowledge, the only other trypsin that has been shown to be resistant to α_1-antitrypsin is mesotrypsin [20]. However, the molecular mechanisms of α_1-antitrypsin resistance of mesotrypsin and SET are clearly different. Mesotrypsin does not recognize the Met358–Ser359 bond in the reactive center loop, and therefore, the enzyme is not inhibited. In contrast to mesotrypsin, we found that SET recognizes and cleaves the Met358–Ser359 bond in the reactive center loop. However, SET is not subsequently trapped by the cleaved inhibitor.

Serpins are thought to be suicide substrate inhibitors of serine proteases. However, some exceptions have been found in which serpins become substrates of serine proteases. For example, C1 inhibitor is a good inhibitor of α-kallikrein at 38 °C, however it becomes mostly the substrate of α-kallikrein at 4 °C [21]. Other studies have also found that some of the serpin variants that have a mutation within the reactive center loop react with proteases principally as substrates [22–24]. Fig. 5 shows the inhibitory and substrate pathways for the reaction between a serpin and protease.

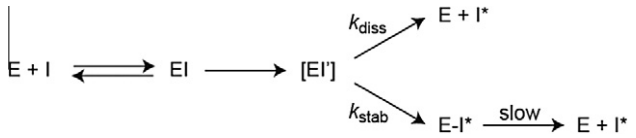

Fig. 5. Reaction scheme between a serine protease and serpin. E is the serine protease, I is the serpin, EI is a noncovalent enzyme–inhibitor complex (Michaelis–Menten complex), [EI′] is an acylenzyme, I* is a reactive site cleaved serpin, and E–I* is a stable and irreversible acylenzyme complex between E and I*. k_{diss} is a rate constant for the dissociation of [EI′], and k_{stab} is a rate constant for the formation of stable and irreversible complex E–I*.

The reaction scheme was originally proposed by Patston and co-workers [21]. According to the reaction scheme, the protease (E) and serpin (I) first associate to form a noncovalent Michaelis–Menten complex (EI), which is followed by the cleavage of a scissile peptide bond in the reactive center loop of the inhibitor, and subsequent formation of acylenzyme (EI′). The acylenzyme (EI′) can go through extensive conformational rearrangement that leads to the formation of a stable and irreversible acylenzyme complex (E–I*) with a rate constant of k_{stab}, or it can dissociate into a free enzyme (E) and reactive site cleaved serpin (I*) with a rate constant of k_{diss} before the conformational rearrangement occurs. According to this reaction scheme, the interaction of SET with α_1-antitrypsin appears to be an extreme example in which the k_{diss} is much greater than k_{stab}. That is, the acylenzyme (EI′) partitions entirely toward substrate. Further studies will be needed to uncover how SET escapes the cleverly placed trap of α_1-antitrypsin. The present study lays the foundation for future studies.

Acknowledgements

This study was supported by NIH grant P30EY-11373 (Visual Sciences Research Center of Case Western Reserve University) and funds from Case Western Reserve University and the Cleveland Foundation. The authors wish to thank Dr. Kulwant S. Aulak (Cleveland Clinic Foundation, Cleveland, OH) for helpful discussions.

References

[1] Yoshida, N., Sasaki, A. and Inoue, H. (1971) An anionic trypsin-like enzyme from *Streptomyces eythreus*. FEBS Lett 15, 129–132.
[2] Nagamine-Natsuka, Y., Norioka, S. and Sakiyama, F. (1995) Molecular cloning, nucleotide sequence, and expression of the gene encoding a trypsin-like protease from *Streptomyces erythraeus*. J Biochem 118, 338–346.
[3] Sakiyama, F. and Kawata, Y. (1983) NMR titration studies of histidine 57 and the [methylene-13C]PMS group in the phenylmethanesulfonyl (PMS) derivative of *Streptomyces erythraeus* trypsin. J Biochem 94, 1661–1669.
[4] Yoshida, N., Sasaki, A. and Inouye, K. (1973) Active site of trypsin-like enzyme from *Streptomyces erythreus.* Specific inactivation by new chloromethyl ketones derived from Na-dinitrophenyl-L-lysine and Na-tosyl-L-arginine. Biochim Biophys Acta 321, 615–623.
[5] Nagata, K. and Yoshida, N. (1984) Interaction between trypsin-like enzyme from *Streptomyces erythraeus* and chicken ovomucoid. J Biochem 96, 1041–1049.
[6] Nagata, K. and Yoshida, N. (1983) Interaction between trypsin-like enzyme from *Streptomyces erythraeus* and Japanese quail ovomucoid. J Biochem 93, 909–919.
[7] Yamane, T., Iwasaki, A., Suzuki, A., Ashida, T., Kawata, Y. and Sakiyama, F. (1995) Crystal structure of *Streptomyces erythraeus* trypsin at 1.9 A resolution. J Biochem 118, 882–894.
[8] Yamane, T., Kobuke, M., Tsutsui, H., Toida, T., Suzuki, A., Ashida, T., Kawata, Y. and Sakiyama, F. (1991) Crystal structure of *Streptomyces erythraeus* trypsin at 2.7 A resolution. J Biochem 110, 945–950.
[9] Kiser, J.Z., Post, M., Wang, B. and Miyagi, M. (2009) *Streptomyces erythraeus* trypsin for proteomics applications. J Proteome Res 8, 1810–1817.
[10] Silverman, G.A. et al. (2001) The serpins are an expanding superfamily of structurally similar but functionally diverse proteins. Evolution, mechanism of inhibition, novel functions, and a revised nomenclature. J Biol Chem 276, 33293–33296.
[11] Baugh, R.J. and Travis, J. (1976) Human leukocyte granule elastase: rapid isolation and characterization. Biochemistry 15, 836–841.
[12] Whisstock, J.C. and Bottomley, S.P. (2006) Molecular gymnastics: serpin structure, folding and misfolding. Curr Opin Struct Biol 16, 761–768.
[13] Huntington, J.A., Read, R.J. and Carrell, R.W. (2000) Structure of a serpin–protease complex shows inhibition by deformation. Nature 407, 923–926.
[14] Pannell, R., Johnson, D. and Travis, J. (1974) Isolation and properties of human plasma alpha-1-proteinase inhibitor. Biochemistry 13, 5439–5445.
[15] Kanamori, A., Seno, N. and Matsumoto, I. (1986) Preparation of high-capacity affinity adsorbents using formyl carriers and their use for low-performance and high-performance liquid affinity-chromatography of trypsin-family proteases. J Chromatogr 363, 231–242.
[16] Nilsson, J., Ruetschi, U., Halim, A., Hesse, C., Carlsohn, E., Brinkmalm, G. and Larson, G. (2009) Enrichment of glycopeptides for glycan structure and attachment site identification. Nat Methods 6, 809–811.
[17] Zhang, Z., Winyard, P.G., Chidwick, K., Murphy, G., Wardell, M., Carrell, R.W. and Blake, D.R. (1994) Proteolysis of human native and oxidised alpha 1-proteinase inhibitor by matrilysin and stromelysin. Biochim Biophys Acta 1199, 224–228.
[18] Desrochers, P.E., Mookhtiar, K., Van Wart, H.E., Hasty, K.A. and Weiss, S.J. (1992) Proteolytic inactivation of alpha 1-proteinase inhibitor and alpha 1-antichymotrypsin by oxidatively activated human neutrophil metalloproteinases. J Biol Chem 267, 5005–5012.
[19] Vissers, M.C., George, P.M., Bathurst, I.C., Brennan, S.O. and Winterbourn, C.C. (1988) Cleavage and inactivation of alpha 1-antitrypsin by metalloproteinases released from neutrophils. J Clin Invest 82, 706–711.
[20] Szepessy, E. and Sahin-Toth, M. (2006) Human mesotrypsin exhibits restricted S1′ subsite specificity with a strong preference for small polar side chains. FEBS J 273, 2942–2954.
[21] Patston, P.A., Gettins, P., Beechem, J. and Schapira, M. (1991) Mechanism of serpin action: evidence that C1 inhibitor functions as a suicide substrate. Biochemistry 30, 8876–8882.
[22] Schechter, N.M., Jordan, L.M., James, A.M., Cooperman, B.S., Wang, Z.M. and Rubin, H. (1993) Reaction of human chymase with reactive site variants of alpha 1-antichymotrypsin. Modulation of inhibitor versus substrate properties. J Biol Chem 268, 23626–23633.
[23] Hood, D.B., Huntington, J.A. and Gettins, P.G. (1994) Alpha 1-proteinase inhibitor variant T345R. Influence of P14 residue on substrate and inhibitory pathways. Biochemistry 33, 8538–8547.
[24] Rubin, H., Plotnick, M., Wang, Z.M., Liu, X., Zhong, Q., Schechter, N.M. and Cooperman, B.S. (1994) Conversion of alpha 1-antichymotrypsin into a human neutrophil elastase inhibitor: demonstration of variants with different association rate constants, stoichiometries of inhibition, and complex stabilities. Biochemistry 33, 7627–7633.